

**AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace all prior versions and listings of claims in the application:

1. (Withdrawn) A sulfur atom-free enzyme protein comprising 18 types of L-amino acid residue: L-alanine; L-aspartic acid; L-glutamic acid; L-phenylalanine; L-glycine; L-histidine; L-isoleucine; L-lysine; L-leucine; L-asparagine; L-proline; L-glutamine; L-arginine; L-serine; L-threonine; L-valine; L-tyrosine; and L-tryptophan.
2. (Withdrawn) The sulfur atom-free enzyme protein according to claim 1 which retains the activity of the original enzyme protein and has oxidation resistance, wherein L-cysteine and L-methionine residues in enzyme proteins comprising 20 types of L-amino acid residue: L-alanine; L-aspartic acid; L-glutamic acid; L-phenylalanine; L-glycine; L-histidine; L-isoleucine; L-lysine; L-leucine; L-asparagine; L-proline; L-glutamine; L-arginine; L-serine; L-threonine; L-valine; L-tyrosine; L-tryptophan; L-cysteine; and L-methionine, are substituted with 18 types of L-amino acid residue: L-alanine; L-aspartic acid; L-glutamic acid; L-phenylalanine; L-glycine; L-histidine; L-isoleucine; L-lysine; L-leucine; L-asparagine; L-proline; L-glutamine; L-arginine; L-serine; L-threonine; L-valine; L-tyrosine; and L-tryptophan.
3. (Withdrawn) The sulfur atom-free enzyme protein according to claim 2 wherein amino acid substitution is carried out by site-directed mutagenesis using synthetic DNA.

FINNEGAN  
HENDERSON  
FARABOW  
GARRETT &  
DUNNER LLP

1300 I Street, NW  
Washington, DC 20005  
202.408.4000  
Fax 202.408.4400  
[www.finnegan.com](http://www.finnegan.com)

4. (Withdrawn) The sulfur atom-free enzyme protein according to any one of claims 1 to 3 wherein the enzyme activity is oxidation-reduction activity or hydrolysis activity.

5. (Withdrawn) The sulfur atom-free enzyme protein according to any one of claims 1 to 4, which retains the activity of dihydrofolate reductase and has oxidation resistance.

6. (Withdrawn) The sulfur atom-free enzyme protein according to any one of claims 1 to 4, which retains the activity of xylanase and has oxidation resistance.

7. (Currently amended) A method of producing a sulfur atom-free enzyme protein having activity greater than or equivalent to an original enzyme protein, wherein the sulfur atom-free enzyme protein is prepared by a combined mutation method comprising the following steps:

(1) preparing a mutant gene by substituting an initiation codon encoding L-methionine in a DNA sequence encoding an enzyme protein ~~consisting of a total length of m amino acids, and having an amino acid sequence comprising n number of sulfur atom-containing amino acids (sulfur-containing amino acids), wherein a position of a sulfur-containing amino acid on the sequence is Ai (i = 1 to n), by with codons encoding any of L-methionine-L-alanine, L-methionine-L-serine, or L-methionine-L-proline~~ ~~codon;[.,.]~~ expressing the prepared mutant gene in a host cell to obtain a mutant enzyme protein;[.,.] measuring enzyme activity of the obtained mutant enzyme protein;[.,.] and

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FARABOW  
GARRETT &  
DUNNER LLP

1300 I Street, NW  
Washington, DC 20005  
202.408.4000  
Fax 202.408.4400  
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selecting the mutant enzyme protein with the highest activity, thereby obtaining a substitution mutant enzyme protein having a substitution A1/MA1;

(2) preparing a mutant gene in which codons a codon encoding a sulfur-containing amino acid[[s]] at another sites a position Ai (i = 2 to n) are is substituted with a codon[[s]] encoding another amino acids among the 18 types of amino acid according to claim 1, any of L-alanine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-asparagine, L-proline, L-glutamine, L-arginine, L-serine, L-threonine, L-valine, L-tyrosine, and L-tryptophan, for a maximum of 18 different substitutions at any Ai;[[.]] expressing the prepared mutant gene in a host cell to obtain a mutant enzyme protein; measuring enzyme activity of the obtained mutant enzyme protein;[[.]] and selecting p-number of mutant enzyme proteins having enzyme activity, thereby obtaining substitution mutants Ai/Bij (j = 1 to p);

(3) selecting a maximum of 3 substitution mutants a maximum of three mutant enzyme proteins having the highest activity, thereby obtaining mutant enzyme proteins having substitutions Ai/Bi1, Ai/Bi2, and Ai/Bi3, wherein activity decreases in order[[.]] Ai/Bi1>Ai/Bi2>Ai/Bi3;>...>Ai/Bip; and

(3) repeating (2) for all Ai; and

(4) selecting substitution mutants having activity in respect of sulfur containing amino acids at all sites Ai (i = 2 to n) in the same manner as (2) and (3) above, preparing a maximum of 3 x (n-i) mutants being all combinations of these mutants with the mutant producing a sulfur atom-free enzyme protein comprising any combination of the substitutions in (2) and (3) with the

FINNEGAN  
HENDERSON  
FARABOW  
GARRETT &  
DUNNER LLP

1300 I Street, NW  
Washington, DC 20005  
202.408.4000  
Fax 202.408.4400  
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substitution of A1/MA1 in (1), wherein a substitution occurs at all Ai;[.]  
measuring the enzyme activity thereof of the sulfur atom-free enzyme protein;[.]  
and obtaining selecting a mutant sulfur atom-free enzyme protein having activity  
greater than or equivalent to that of the original enzyme protein.

8. (Currently Amended) A method of producing a sulfur atom-free enzyme protein having activity greater than or equivalent to an original enzyme protein, wherein the sulfur atom-free enzyme protein is prepared by a stepwise mutation method comprising the following steps:

(1) preparing a mutant gene by substituting an initiation codon encoding L-methionine in a DNA sequence encoding an enzyme protein ~~consisting of a total length of m amino acids, and having an amino acid sequence comprising n number of sulfur atom-containing amino acids (sulfur-containing amino acids), wherein a position of a sulfur-containing amino acid on the sequence is Ai (i = 1 to n), by with codons encoding any of L-methionine-L-alanine, L-methionine-L-serine, or L-methionine-L-proline codon;~~; expressing the prepared mutant gene in a host cell to obtain a mutant enzyme protein;[.] measuring enzyme activity of the obtained mutant enzyme protein;[.] and selecting the mutant enzyme protein with the highest activity, thereby obtaining a substitution an A1/MA1 mutant A1/MA1;

(2) preparing a mutant gene in which codons a codon encoding a sulfur-containing amino acid[[s]] at A2 of the A1/MA1 mutant of (1) is substituted with a codon encoding another amino acid any of L-alanine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-glycine, L-histidine, L-

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GARRETT &  
DUNNER LLP

1300 I Street, NW  
Washington, DC 20005  
202.408.4000  
Fax 202.408.4400  
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isoleucine, L-lysine, L-leucine, L-asparagine, L-proline, L-glutamine, L-arginine, L-serine, L-threonine, L-valine, L-tyrosine, and L-tryptophan, for a maximum of 18 different substitutions at A2; being one of the 18 types of amino acid according to claim 1, expressing the prepared mutant gene in a host cell to obtain a double mutant;[[.]] measuring enzyme activity of the obtained double mutant enzyme protein; and selecting a maximum of 3 triple double mutants with the highest activity;

(3) preparing a mutant gene in which a codon encoding a sulfur-containing amino acid at each A3 of the obtained double mutants is substituted with a codon encoding another amino acid any of L-alanine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-asparagine, L-proline, L-glutamine, L-arginine, L-serine, L-threonine, L-valine, L-tyrosine, and L-tryptophan, for a maximum of 18 different substitutions at A3; being one of the 18 types of amino acid according to claim 1, expressing the prepared mutant gene in a host cell to obtain a triple mutant;[[.]] measuring enzyme activity of the obtained triple mutant enzyme protein; and selecting a maximum of [[3]] three triple mutants with the highest activity; and

(4) repeating the stepwise substitution of codons encoding sulfur containing amino acids at each remaining Ai (i = 4 to n) in the same manner as described above in (2) and (3), wherein positions Ai-An are substituted in any order; preparing a quadruple mutant, ..., multiple number of n mutant, inspecting measuring enzyme activity of last multiple number of n mutant, a sulfur atom-free enzyme protein obtained upon substitution of An; and preparing selecting a

FINNEGAN  
HENDERSON  
FARABOW  
GARRETT &  
DUNNER LLP

1300 I Street, NW  
Washington, DC 20005  
202.408.4000  
Fax 202.408.4400  
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mutant sulfur atom-free enzyme protein having activity greater than or equivalent to that of the original enzyme protein.

9. The process for producing a sulfur atom-free enzyme protein prepared by the stepwise mutation method according to of claim 8 wherein A1-An are substituted in order of position on the amino acid sequence the order of stepwise mutation sites is according to any one of (n! types) permutations and combinations of A1, A2, ..., An.

10. (Currently amended) A process for producing a sulfur atom-free enzyme protein using a combination of a combined mutation method and a stepwise mutation method, wherein the method comprises: in an enzyme protein consisting of a total length of m amino acids and comprising n number of sulfur containing amino acids, in which a site of a sulfur containing amino acid on the sequence is Ai (i = 1 to n), a process according to claim 7 is adopted at k number of sites and a process according to claim 9 is adopted at remaining n - k number of sites.

(1) preparing a mutant gene by substituting an initiation codon encoding L-methionine in a DNA sequence encoding an enzyme protein having an amino acid sequence comprising n number of sulfur atom-containing amino acids, wherein a position of a sulfur-containing amino acid to be substituted is designated as Ai (i = 1 to k, k ≤ n), with codons encoding any of L-methionine-L-alanine, L-methionine-L-serine, or L-methionine-L-proline; expressing the mutant gene in a host cell to obtain a mutant enzyme protein; measuring enzyme activity of the mutant enzyme protein; and selecting the protein with the highest activity, thereby obtaining a mutant enzyme protein having a substitution A1/MA1;

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FARABOW  
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1300 I Street, NW  
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(2) preparing a mutant gene in which a codon encoding a sulfur-containing amino acid at a position Ai (i = 2 to k) is substituted with a codon encoding any of L-alanine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-asparagine, L-proline, L-glutamine, L-arginine, L-serine, L-threonine, L-valine, L-tyrosine, and L-trypotophan, for a maximum of 18 different substitutions at any Ai; expressing the mutant gene in a host cell to obtain a mutant enzyme protein; measuring enzyme activity of the mutant enzyme protein; and selecting a maximum of three mutant enzyme proteins having the highest activity, thereby obtaining mutant enzyme proteins having substitutions Ai/Bi1, Ai/Bi2, and Ai/Bi3, wherein activity decreases in order Ai/Bi1>Ai/Bi2>Ai/Bi3; and

(3) repeating (2) for all Ai;

(4) producing a mutant enzyme protein comprising any combination of substitutions as in (1), (2), and (3), wherein a substitution occurs at all Ai; measuring enzyme activity of the mutant enzyme protein; and selecting a maximum of three mutant enzyme proteins having the highest activity; and

(5) producing a sulfur atom-free enzyme protein by subjecting the mutant enzyme proteins selected in (4) to stepwise substitution of the remaining n - k number of sulfur-containing amino acids as in claim 8; measuring the enzyme activity of the sulfur atom-free enzyme protein; and selecting a sulfur atom-free enzyme protein having activity greater than or equivalent to that of the original enzyme protein.

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